ABSTRACT

A method for obtaining a mixture of heterogenous short double-stranded RNA molecules suitable for use in gene silencing (hsiRNA) by subjecting large double-stranded RNA to enzymatic cleavage under specified conditions. The resulting mixture consistently includes enhanced representation of fragments having a size of 21-22 nucleotides absent any fractionation step. The fragments contain sequences that collectively span the entire length of the large double-stranded RNA from which they are derived. Double-stranded RNA with sequences that individually represent segments of a target mRNA may be analyzed using the methods described herein to identify the most active subset of hsiRNA fragments or individual siRNA fragments for achieving gene silencing for any gene or transcribed sequences. A method is additionally provided for preparing and cloning DNA encoding selected siRNA, hsiRNA mixtures or hairpin sequences to provide a continuous supply of a gene silencing reagent derived from any long double-stranded RNA.